

RHYTHM VARIABLES AS TAXONOMIC CHARACTERS IN ANTS

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A survey of many species of ants in the field indicates a generic difference in the hours of flight and of worker aboveground activity (McCluskey 1973, 1974). Further, species within the same genus may differ consistently (Holldobler 1976; Lévieux 1977; Whitford and Ettershank 1975). Would the same be true if the habitats of the various species were made identical? The present paper reports a beginning laboratory test of this question in worker ants. Secondly, it uses multivariate analysis to examine rhythm and other laboratory-type variables for their potential as taxonomic characters. A preliminary report was given by McCluskey and Soong (1978).

METHODS AND MATERIALS

The species observed were: *Pogonomyrmex californicus* (Buckley), *Pogonomyrmex rugosus* Emery, *Veromessor andrei* (Mayr), and *Veromessor pergandei* (Mayr) in the tribe Myrmicini of Myrmicinae; and *Formica pilicornis* Emery and *Myrmecocystus mimicus* Wheeler in the tribe Formicini of Formicinae. The ants were all collected May 24 and 25, 1973, near Loma Linda or Colton, San Bernardino County, California, at an elevation of about 350 m.

Each group of ants was placed in a clear plastic refrigerator dish 9 cm in diameter by 3 cm deep. Sugar water was provided by a cotton-plugged tube hanging down through the lid, and constant moisture by a wick of dental cotton projecting up from water in a like dish nested underneath. A clear plastic (Tygon) tube 8 cm long led to a dish of the same type to serve as a dry arena. The nest dish was totally covered by heavy black paper, while the tube and arena were fully exposed to the light.

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There were five of these nest assemblies per species (except only one for *M. mimicus*), with 30 ants in each. The 26 arenas were lined up side by side, the first replicate of each species, then the second of each, etc. Heating tape was stretched alongside the whole row of arenas so that it was about 1 cm from one side of each arena; it provided constant heat throughout the experiment.

The ants were kept in a constant temperature room. The nest dishes averaged $24.3^{\circ}\text{C} \pm 0.5^{\circ}$. The hot side of the arenas was kept at least $10^{\circ} (\pm 1^{\circ})$ hotter than this. Twelve hours of fluorescent light (2000 lux) was alternated with 12 hours of darkness (the last 2 nights there was dim incandescent light [15 lux] to permit observations).

The ants were installed May 25 and observations began May 27. Each hour during the light periods of May 27–31, and also during the 2 nights beginning May 30 and 31, the number in the hot half of the arena, in the warm half of the arena, and in the tube were counted visually. In addition, the total number active (moving) in tube and arena were counted.

Except where noted, an arc sine transformation was used, treating the count as a proportion of the 30 ants available (Dixon and Massey 1969). Then the transformed counts for the 3 days May 27–29, when the ants were in the best condition, were averaged for each replicate. Finally the hourly observations were fitted to a 24-hour cosine-curve for determination of the best-fit peak (acrophase) and other rhythm parameters.

Variables evaluated

The fitting method used (Halberg et al. 1972) provides the acrophase, the level (average), the amplitude, and the sinusoidality (probability of sine shape) of the fitted rhythm. Each of these four parameters (dependent variables) could serve as a potential character, and each was applied to every one of the following four types of assay (Table 1).

Listed first are the variables related to the total number of ants out (i.e., in tube and arena) at each hour (TOTAL OUT). This would represent choice of an area exposed to alternating light and dark, as well as drier and warmer than the dark humid next dish.

The second type of assay was the number of ants in the hot half of the arena (TEMPERATURE). This represents the extreme distance out from the nest dish, and/or the preference or tolerance of high temperature.

The third, POSITION, was calculated as $(.80 \times \text{total no. out} - \text{no. in tube}) / (\text{total no. out} - \text{no. in hot half of arena})$. It is an index of how far out into the warm (i.e., nearest) half of the arena one would have to go to encompass 80% of the ants which were out of the nest at the time (50% would not work, because too often the 50% line was down in the tube rather than out in the arena). This position assay may be illustrated by sample calculations: If all the ants were in the warm half of the arena, the 80% point would be 80% of the way out through the warm half. If 20% were in the warm half and 80% in the tube, the 80% point would be 0% of the way through the warm half. If 80% were in the warm half and 20% in the hot half, the 80% point would be 100% of the way out through the warm half.

The fourth, ACTIVITY, was the proportion active of the total number out.

The fitting involved above should increase the information content in any given variable. How important is this for taxonomic discrimination? To help answer the question, single "hourly" (i.e., nonfitted) variables were also tested (Table 1), each being simply the value for a particular hour.

Table 1. (see following pages)

Means of variables examined for taxonomic value, averaged over days (May 27-29 except where noted) and replicate nests for each species (abbreviated by initial of genus and species). TOTAL OUT and TEMPERATURE based on arc sine transformation of count; POSITION based on raw count; ACTIVITY based on log of proportion active. *Sinusoidality* represented by log of F statistic, *Acrophase* by clock hour (decimal form: e.g., 15.8 = 3:48 PM; angular avg), and *Level* and *Amplitude* by number of ants (except for POSITION, where *Level* and *Amplitude* represent distance into arena—see text); these first 4 variables under each type of assay are all derived by least-squares cosine-fitting (Halberg et al., 1972). The next 6 variables under TOTAL OUT are: *Shift of Acrophase* (no. of h change in acrophase from May 27 to May 29); *Rise* (h at which count passed up through avg [*Level*] for the day); *Shift of Rise* (no. of h change from May 27 to May 29); *Above Average* (no. of h above avg for the day, May 30-31, when observed all 24 h); *Light-on Difference* (between count immediately after lights on and 2d count several min later, May 28-30); *Fluctuation* (no. of runs, where "run" = series of hourly values each having same sign of change from preceding value). Corresponding to latter is *Fluctuation* under POSITION. Finally, under each type are listed the hourly values, unprocessed except for initial transformation and averaging (ACTIVITY 0700 omitted because could not observe all replicates simultaneously at the moment lights came on). F based on 5,20 degrees of freedom (slightly less for some variables with missing observations); * = $P < .05$, ** = $< .01$, *** = $< .001$.

TABLE I

Variable	Species						F
	P.c.	P.r.	V.a.	V.p.	F.p.	M.m.	
TOTAL OUT							
Sinusoidality	1.79	1.81	1.74	1.62	1.45	1.83	.9
Level	72	83	83	45	65	56	4.8**
Amplitude	25	32	41	28	23	19	1.5
Acrophase	15.8	18.0	.7	22.5	23.4	20.8	23.3***
Shift of acro.	1.4	4.0	.9	.3	2.1	2.8	2.4
Rise	10.7	11.9	18.4	16.0	17.1	14.6	15.1***
Shift of rise	1.3	3.9	.6	-1.0	1.3	2.2	4.0**
Above average	15.0	12.6	12.7	9.2	11.4	13.0	6.4**
Light-on diff.	28.2	40.2	40.8	6.2	41.6	10.5	4.2**
Fluctuation	16.2	15.4	13.0	10.8	18.2	16.0	4.3**
0700	59	62	85	25	60	41	5.6**
0800	60	55	61	22	47	34	2.7
0900	64	61	55	20	44	30	2.9*
1000	70	65	49	20	45	45	2.9*
1100	74	74	48	21	44	41	4.2**
1200	81	84	48	21	43	42	6.4**
1300	89	91	47	23	44	41	8.7***
1400	92	99	45	24	46	50	11.8***
1500	96	106	49	31	52	59	15.0***
1600	97	110	57	45	58	66	11.8***
1700	95	109	67	55	65	66	5.5**
1800	93	111	79	57	70	69	7.0***
1900	86	110	88	61	72	72	4.5**
POSITION							
Sinusoidality	1.78	.92	.79	1.18	.44	.18	9.3***
Level	89	69	70	30	3	59	9.8***
Amplitude	42	12	13	13	14	9	3.9*
Acrophase	20.6	18.7	3.4	11.0	14.5	20.1	5.2**
Fluctuation	19.6	20.6	17.7	11.0	23.7	22.0	6.3**
0700	57	48	74	45	1	2	5.4**
0800	46	60	78	47	0	87	3.4*
0900	52	62	81	31	1	73	3.8*
1000	47	66	60	31	8	41	1.8
1100	49	63	62	43	14	64	1.2
1200	61	70	61	43	9	49	2.2
1300	66	67	58	44	19	54	1.4
1400	77	73	61	46	19	62	2.1

TABLE 1 (CONTINUED)

1500	109	70	56	40	19	45	4.5**
1600	110	77	55	36	14	60	7.8***
1700	118	78	62	28	18	68	20.7***
1800	116	84	56	24	6	55	12.5***
1900	121	83	62	21	9	90	14.3***
TEMPERATURE							
Sinusoidality	1.72	1.36	.58	.29	.24	1.00	11.9***
Level	31.7	24.5	14.0	11.5	11.7	19.7	5.6**
Amplitude	20.8	14.0	4.1	1.0	.8	9.6	6.9***
Acrophase	19.2	21.8	1.4	1.8	.5	23.1	2.1
0700	14	13	18	13	12	10	.8
0800	12	12	12	12	12	15	.4
0900	14	12	11	10	10	13	3.5*
1000	14	12	10	10	11	10	3.2*
1100	17	11	10	10	11	13	10.8***
1200	23	11	10	10	11	10	20.9***
1300	30	15	11	10	11	10	13.5***
1400	33	17	11	11	11	10	6.3**
1500	49	22	10	12	10	13	13.2***
1600	49	25	11	10	11	20	14.5***
1700	52	29	13	12	13	20	10.3***
1800	51	32	12	11	12	15	9.0***
1900	47	35	12	10	11	31	7.4***
ACTIVITY							
Sinusoidality	.75	.78	.55	.84	.63	.11	1.3
Level	.91	1.09	.93	.53	.94	.60	4.0**
Amplitude	.24	.33	.65	.39	.37	.18	3.0*
Acrophase	20.6	19.7	.4	22.4	.0	12.5	3.4*
0800	77	82	90	49	61	42	.8
0900	71	91	58	18	59	114	2.8*
1000	58	68	26	8	62	79	3.1*
1100	55	84	6	0	75	39	10.7***
1200	88	90	18	0	54	82	9.2***
1300	83	103	29	12	69	84	7.6***
1400	67	111	14	23	50	81	4.9**
1500	103	124	44	43	84	69	4.3**
1600	103	138	103	64	83	85	3.5*
1700	106	142	106	73	92	73	5.5**
1800	103	134	67	50	113	23	8.0***
1900	107	133	73	49	117	80	6.2**

Several miscellaneous variables are also shown in Table 1, such as "shift of acrophase." They and the fitted variables will be referred to as "processed" variables to distinguish them from the "hourly" ones.

Multivariate analysis of variance (MANOVA) and multidiscriminant analysis were used for comparison of species by groups of variables. These methods apply even where variables are highly correlated, which many of those here certainly are. On the basis of known group membership, discriminant analysis emphasizes variables that have a high ratio of among-group to within-group variation. Thus the variables are weighted so as to discriminate optimally between the groups (here species). An introduction to these methods is given by Cooley and Lohnes (1971), and application to behavioral research, by Pimentel and Frey (1978).

RESULTS

Daytime observations

For both species of *Pogonomyrmex* the total number out rose in early midday and remained high through the afternoon (Figure 1). For the other species the number rose in the afternoon and continued rising through the end of the light period.

In *V. pergandei* and *F. pilicornis* few ants ever came out as far as the arena; the rhythmic count was made up largely of those in the tube leading to the arena (Figure 1). In *V. andrei* the proportion in the tube was relatively high at the first and last of the light period. The proportion reaching the hot side of the arena was the greatest in *P. californicus*.

For most of the analyses, only these daytime observations were used.

24-hour observations

After 3 days very dim lights were left on at night, permitting counts then. It was now 6 days after collection from the field, and some of the ants were dead or else came out into the tubes or arenas less frequently. Nevertheless the patterns for the "day" part of the cycle were similar to before. And now it could be seen that all six species were prominently out in the arenas during the night.

That this nocturnal behavior might be explained by the constant high temperature is suggested by field observations. Tevis (1958) found that nocturnal foraging of *V. pergandei* is rare, but could occur if temperatures are higher than normal; Wheeler and Rissing

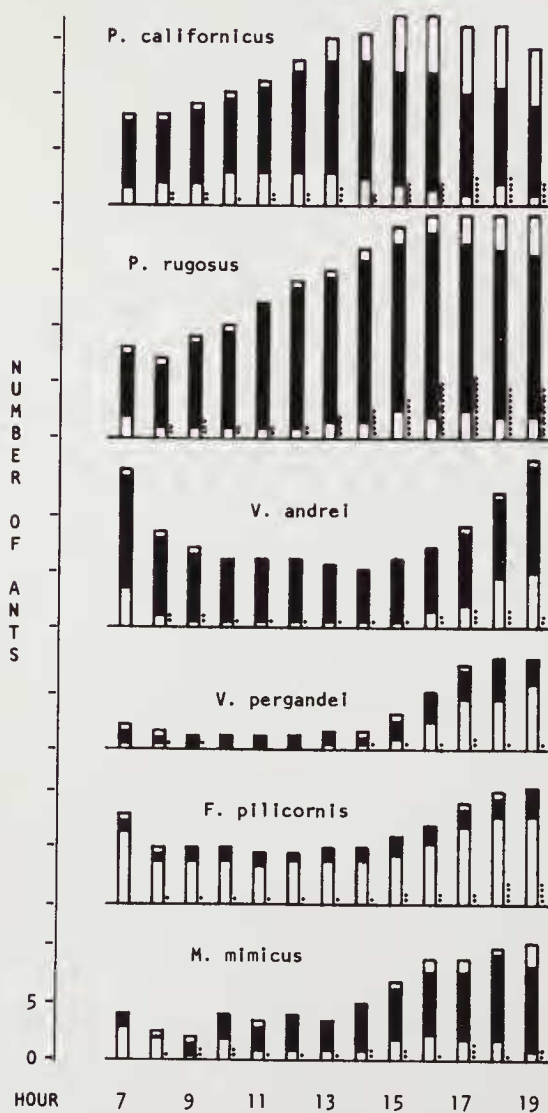


Figure 1. Comparative time patterns during light part of light-dark cycles. Histograms based on raw counts averaged over the 3 days (May 27-29) and 5 replicate nests (1 nest for *M. mimicus*) for each species. Each bar represents total number of ants out at a given observation: bottom part, tube count; middle (shaded), warm half of arena; top, hot half of arena. Dotted line to right of bar represents number active of total number out (omitted for 0700).

(1975) found it to be common in the summer. McCluskey (1963) reported foraging in *V. andrei* on warm nights. *P. californicus* sometimes works at night (Cole 1932), and *P. rugosus* does so when warm (Whitford and Ettershank 1975).

Evaluation of variables as taxonomic characters

By virtue of the replicates it was possible to test each variable for species difference by one way analysis of variance, or in the case of phase variables, by the circular distribution test of Watson and Williams (1956) (Table 1). The differences are significant for 42 of the 51 hourly and 18 of the 23 processed variables, suggesting value as species characters. The hourly variables for the last third of the day are more discriminating than those earlier.

Thus most of the individual variables distinguish species at least in a general way. As a group would they distinguish between the members of each pair of species? Multidiscriminant analysis was used to answer this (somewhat as Brown and Shipp, 1977, did to evaluate wing morphometric characters).

M. mimicus, with only one replicate, was omitted. MANOVA indicates a significant ($P < .01$) difference among the other five species, whether based on processed or on hourly variables. Therefore it is appropriate to use multidiscriminant analysis for further examination of the differences. For the set of processed variables, Figure 2 shows the maximum discrimination possible in two-dimensional space. The replicates are well segregated into their respective species. The same was true if based on either of the two stratified subsets of 17 hourly variables tried (not shown). Further analysis is not attempted here because of small sample size.

The method was checked by deliberately assigning half the replicates initially to incorrect species. The variables then failed to segregate the replicates well into the artificial species, or even to show a significant species difference by MANOVA.

Later experiment

As laboratory projects in a comparative physiology course, the same species of *Pogonomyrmex* and *Veromessor* were compared simultaneously as before, but the conditions were different. Most notably, the season was October instead of May; and the temperature was cycled between 20° C at night and 30° (35° in hottest part of arena) at midday, instead of being held constant.

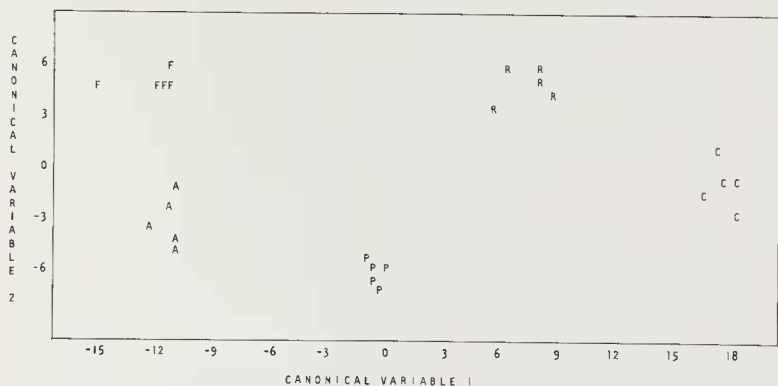


Figure 2. The first two canonical axes, based on all 17 processed variables for which every one of the 25 cases (5 species $\times$ 5 replicates) was represented, and for which (phase variables) distribution was across few enough hours to assume linearity. The first axis represents the greatest possible difference between species, and the second, the greatest remaining difference orthogonal to the first. Output from the BMDP7M program, run so as to bypass the stepwise feature (as recommended by Pimentel and Frey, 1978). A = *V. andrei*; C = *P. californicus*; F = *F. pilicornis*; P = *V. pergandei*; R = *P. rugosus*.

The results again suggest taxonomic differences in rhythm. The cosine-fitted acrophases of number out differed ($P < .01$) among the four species.

DISCUSSION

The study conditions were highly artificial—colony fragments (workers only, and in small number), no soil in the nest, temperature high all 24 hours, etc. The fact that the rhythms were nevertheless striking indicates that at least some of the natural field conditions (e.g., temperature cycle) are not necessary for such behavior.

But the important thing was that the six species were now all under the same conditions. Evidently various species manifest different patterns of rhythm even though in identical habitat; or to put it another way, it is not necessarily differences in field habitat that are responsible for the differences in rhythm. For a laboratory example of phase or pattern difference in another insect group, see Hardeland and Stang's (1973) comparison of 40 species of *Drosophila*.

Many location and activity variables were tested in the six species for their potential as taxonomic characters, including cosine-fitted rhythm parameters as well as simple hourly values (Table 1). For most of them the species difference was significant.

By contrast with the fitted-rhythm and other processed variables, the hourly variables taken singly can indicate only the level (of activity or location) at a given hour. In the aggregate they become more meaningful. For example, scanning the hourly *P* values in Table 1 indicates one time of day to be more important than another in distinguishing species. Or more formally, a multivariate approach can compare in terms of the whole hourly pattern. MANOVA confirmed the overall species difference, and multidiscriminant analysis made it possible to discriminate each species from the others. It should be noted again that the purpose here is to examine these variables as taxonomic characters, rather than to develop a classification.

Nearly every one of the processed variables was abstracted from all the hourly values of a given type of assay, and hence should have high information content. So it is noteworthy that the groups of hourly variables seemed to distinguish species about as well as the group of processed ones. However, if some of both kinds were included in the same analysis, the most highly weighted were processed variables. most highly weighted were processed variables.

How might the list in Table 1 be compared with catalogues of ant behavior, such as that of Wilson (1976)? In the first place, the variables reported here were population rather than individual phenomena. Secondly, many of them are derived rhythm parameters (e.g., acrophase or amplitude) of certain items of behavior, rather than the behavior itself. The hourly variables, on the other hand, do represent behavior more directly (e.g., the number active or the number in the hot location, at a given point in time). Any comprehensiveness of catalogue (e.g., for choice of heat or light or location in the nest assembly) would require more habitat complexity, and preferably field studies as well. But such comprehensiveness is needed to facilitate behavioral comparisons at species or higher levels (Wilson 1976).

In summary, this study suggests a variety of rhythm-related characters that might be observed in laboratory or field, whether single hourly values, composite hour-by-hour patterns, or fitted rhythm parameters.

SUMMARY

In the field the time of day of certain types of behavior distinguishes various species and genera. Would the same be true if their habitats were made identical? And could rhythm-related variables in general be regarded as taxonomic characters? Groups of workers of *Pogonomyrmex californicus*, *P. rugosus*, *Veromessor andrei*, *V. pergandei*, *Formica pilicornis*; and *Myrmecocystus mimicus* were studied simultaneously in constant temperature and alternating light and dark. A hot dry area and a dark humid area were available in each nest for choice at all hours. This permitted different types of position or activity assays and the calculation of several cosine-fitted rhythm parameters for each. These as well as simple hourly values served as variables. For most of the variables tested there was a significant species difference. With multidiscriminant analysis it was possible to go beyond this general species difference and segregate the replicates of each species from those of other species; either the hourly or the more highly processed variables were adequate here. This study suggests a variety of rhythm-related characters that might be observed in laboratory or field.

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